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Electrogenicity at the secondary quinone acceptor site of cyanobacterial photosystem II

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Abstract

Flash-induced generation of the electric potential difference ($\Delta\psi$) by a direct electrometrical method was studied in *Anacystis nidulans* photosystem II-containing proteoliposomes associated with a phospholipid-impregnated collodion film. Besides a rapid phase of $\Delta\psi$ generation corresponding to charge separation between P680 and Q_A, an additional electrogenic phase with a characteristic time of 0.27 ms at pH 7.0 was observed after the second laser flash. The maximal amplitude of this phase was approx. 4% of that related to the $P680^+Q_A^-$ formation. The sensitivity of this phase to DCMU, the flash-number dependence of its amplitude as well as the amplitude and the rate constant pH-dependences, indicate that it is due to the dismutation of Q_A^- and Q_B^- and to subsequent protonation of a doubly reduced plastoquinone Q_B^{2-}.

Key words: Photosystem II; Proteoliposome; Electrogenicity; Plastoquinone; *Anacystis nidulans*

1. Introduction

The ability of the photosystem (PS) I and II reaction center (RC) complexes from plants, green algae and cyanobacteria to generate a photovoltage has been shown by various methods [1–10].

The investigation of the primary electrogenic reactions of PS II RC in pea chloroplasts showed that the charge separation between the primary electron donor, P680, and the intermediary electron acceptor, pheophytin, accounts for approx. 1/2 to 2/3 of the total charge separation between P680 and the primary quinone acceptor (Q_A) [6]. The electron transfer from Y_Z (Tyr-161 of the D1 protein) to photooxidized P680 is also electrogenic, as demonstrated in PSII particles from spinach [7] and from the thermophilic cyanobacterium *Synechococcus elongatus* [8]. The data obtained through the observation of electroluminescence changes indicate that an electrogenic reaction related to electron transfer from the Mn-cluster of the oxygen-evolving complex to Y_Z spans 5% of the membrane core [9]. There is some evidence to suggest that proton transfer to the doubly reduced secondary quinone acceptor (Q_B) contributed with $\leq$ 5% of the primary charge separation voltage in PSII reaction centres from spinach [10].

In the present study we investigated the photoinduced charge transfer reactions in the quinone acceptor complex of O_2-inactive PSII particles from the mesophilic cyanobacterium *Anacystis nidulans*.

2. Materials and methods

PSII particles were prepared from *Anacystis nidulans* by the procedure described by Barsky et al. [4] with minor modifications. After the LDAO treatment and subsequent centrifugation at 100,000 × *g* for 60 min, the supernatant was treated according to Corrie et al. [11] with 10% (w/v) PEG_{6000} to precipitate the PSII particles. The particles were kept frozen at −80°C until use.

Proteoliposomes were prepared according to [8].

Measurements of photoelectric activity by PSII adsorbed onto the surface of asolectin-impregnated collodion film were done and the kinetic data were processed as in [12]. Redox potential titrations were performed using platinum and Ag/AgCl electrodes at pH 7.0 under anaerobic conditions [8]. The following mediators were used (10 μM): diaminodurene, 1,2-naphthoquinone-4-sulphonate, 1,2-naphthoquinone, menadione, Indigo tetrasulphonate, Indigo disulphonate, antraquinone sulphonate, Neutral red, 1 μM Methylene blue, 1 μM methyl viologen, 5 μM phenasine methosulphate.

3. Results and discussion

The reduction of Q_B in RC of PSII requires two consecutive photoreactions. The Q_A is reduced photochemically to the semiquinone state Q_A^- and it in turn reduces a secondary PQ molecule to the semiquinone state, Q_B^-. When Q_A^- is photoreduced a second time, it reduces Q_B^- to the fully reduced plastoquinol state; protons are taken up in this process also, in a manner that is still unclear.

Fig. 1A shows the photoelectric responses induced by the first (trace 1) and second (trace 2) laser flashes of O_2-inactive PSII-containing proteoliposomes associated with a phospholipid-impregnated collodion film. PSII was found to be incorporated into the liposome membrane in such a fashion that P680, a primary electron donor, was located on the outer side of the membrane. Therefore the sign of the photoelectric responses was always negative inside the vesicles.

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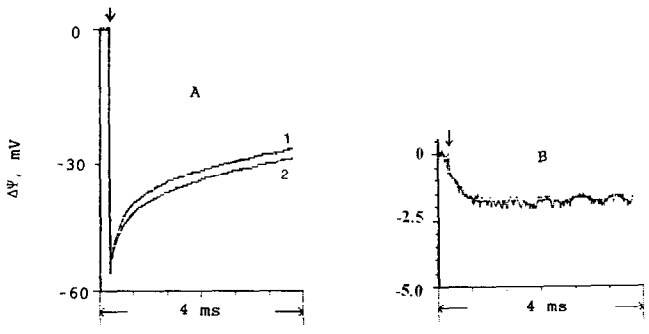

Fig. 1. Generation of an electrical potential by *A. nidulans* PSII-containing proteoliposomes induced by the first (trace 1) and the second (trace 2) laser flashes (A), and 2nd minus 1st flash difference (B). The incubation medium contained 50 mM HEPES, pH 7.0. 5 mg/ml of decylplastoquinone were added to the decane solution of the phospholipids used to impregnate the collodion film. The vertical arrows indicate laser flashes. The second flash was given 1 s after the first.

As we have shown previously [8], the rapid $\Delta\psi$ generation (< 0.1 μs, resolution limit of the apparatus) is due to electron transfer from P680 to the primary quinone acceptor Q_A. This conclusion was confirmed by the results of redox titration of the photoresponse, the amplitude of which decayed under the equilibrium reduction of Q_A (Fig. 2). The experimental points fit the theoretical Nernst curve for one-electron transfer with midpoint potential (E_m) of −100 mV, which agrees with the value of E_m for Q_A in PSII from thermophilic cyanobacteria [8]. It can be seen from Fig. 1B that the second flash gives a small additional electrogenic phase. The difference between the two kinetics is more distinctly seen in Fig. 1B. The amplitude of the difference between traces (2−1) depends on the time interval between the two flashes and reaches a maximal value within 2 s (not shown). The maximal amplitude of the phase with a characteristic rise time of 0.27 ms (pH 7.0) constituted approx. 4% of the fast electrogenic phase associated with the formation of $P680^+Q_A^-$. This phase was completely abolished by addition of 3-(3,4-dichlorophenyl)-1,1-dimethyl-urea (DCMU), an inhibitor of electron transfer between Q_A and Q_B (not shown).

The pH dependence of the DCMU-sensitive electrogenic phase amplitude is presented in Fig. 3A. In the pH range from 6.0 to 8.5 the amplitude of the observed electrogenic reaction comprises approx. 4% of the ampli-

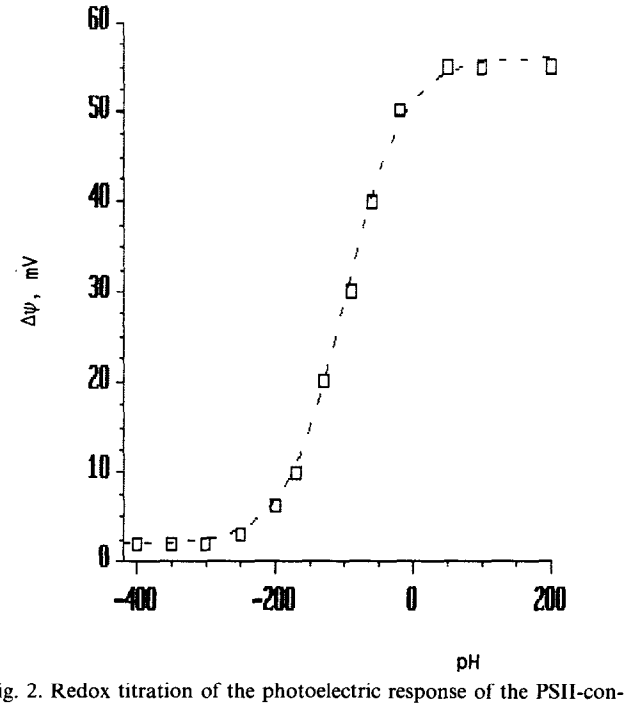

Fig. 2. Redox titration of the photoelectric response of the PSII-containing proteoliposomes. The titration was carried out at pH 7.0. The dashed curve represents one electron Nernst behavior for E_m of −100 mV.

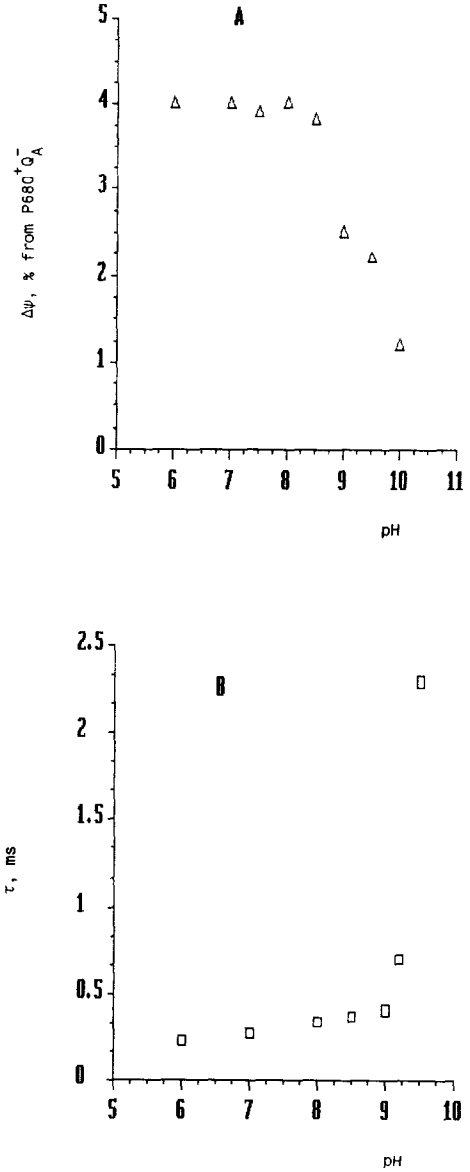

Fig. 3. Effect of pH on the amplitude (A) and the rise-time (B) of the second flash-induced electrogenic phase. Conditions were as in Fig. 1 except that 15 mM of MES, HEPES, Tris and CAPS, each, were added.

tude due to formation of $P680^+Q_A^-$ and decreases under more alkaline pH values. The pH dependence of the rise time of the second flash-induced electrogenic phase is shown in Fig. 3B. As can be seen, pH has only a small effect on the kinetics of the phase at pH 6.0–9.0 while the rise time increase at pH > 9.0 was rather more evident. The profile of these dependences is similar to that observed for the analogous phase of purple and green bacteria [13–16].

Thus the sensitivity of this phase to DCMU, the flash number and pH-dependence of its amplitude and rise time indicate that this is due to protonation of Q_B^{2-}.

Kinetic measurements of $\Delta\psi$ generation in chromatophores and RC-containing proteoliposomes of purple and green bacteria showed that the protonation of the Q_B^{2-} gives rise to an electrogenic phase which contributes as much as 15–20% of the total $\Delta\psi$ generation by the flash [13–16].

The data presented suggest that the small electrogenicity in RC PSII associated with the protonation of Q_B^{2-} may be due to smaller distances between the Q_B and the membrane surface and/or to larger dielectric constants in PSII than in RCs of purple bacteria. However, we can not exclude the possibility that in the preparations of PSII used in the present work, the reconstruction of Q_B function in the presence of excess decyl-plastoquinone is smaller than in RCs from purple or green bacteria [13–16].

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